Animal Behaviour 203 (2023) 1–9

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Active background selection facilitates camouflage in shore crabs, *Carcinus maenas*

Laura Twort, Martin Stevens*

Centre for Ecology and Conservation, University of Exeter, Penryn Campus, U.K.

ARTICLE INFO

Article history:
Received 16 January 2023
Initial acceptance 8 March 2023
Final acceptance 5 May 2023

MS. number: 23-00026

Keywords:
background matching
behavioural choice
camouflage
crypsis
shore crab

Camouflage plays a significant role in preventing and facilitating predation. A common method used by many species to avoid detection is to match aspects of the visual background. Behaviour can constitute a valuable component of camouflage by enabling animals to choose appropriate substrates, yet how widespread this is remains relatively underexplored. Through a series of substrate choice experiments we tested whether the highly phenotypically diverse common shore crab shows substrate preferences, and whether preferences reflect choices that actively improve individual camouflage. Using image analysis, we compared brightness and colour metrics of crabs to their chosen versus alternative substrates. Crabs tended to choose substrates with a brightness that better matched their own appearance. However, choices depended on the exact backgrounds offered, for example with crabs preferring backgrounds resembling native rock pool colour patterns over those resembling mudflats, but showing little difference in choice between red and green substrates. The results help explain observations that shore crabs and other animals show phenotype–environment associations at a microscale and demonstrate how individuals can maintain camouflage in highly variable visual environments. Our study shows that substrate preferences can be a key route to enabling camouflage in a broad spectrum of species.

Predation is one of the most significant selection pressures driving evolution in many different species (Endler, 1984; Stevens & Merilaita, 2009; Stevens & Ruxton, 2019). Consequently, animals have evolved numerous strategies to avoid being attacked. Camouflage is arguably the most widespread and prevalent defence, encompassing a range of strategies for avoiding detection or recognition observed across a diverse range of taxonomic groups (Endler, 1978; Briffa et al., 2008; Merilaita & Stevens, 2011; Rowland et al., 2007; Ruxton et al., 2004; Stevens et al., 2015). In most cases, successful visual camouflage involves matching general features of the environment, often termed background matching. Correspondingly, many species have evolved appearances that resemble their local habitat over many generations (e.g. Kettlewell, 1955; Walton & Stevens, 2018; Rosenblum, 2006). Alternatively, a wide range of species can change appearance over different timescales to match their environment (reviewed in Duarte et al., 2017), also leading to phenotype–environment matches (e.g. Boratyński et al., 2017; Stevens et al., 2014a; Todd et al., 2006).

One of the major challenges for successful camouflage is that most environments are heterogeneous in appearance in space and time, making effective matching problematic when animals move or when the habitat changes (Caro et al., 2016; Magellan & Swartz, 2013; Michalis et al., 2017). In species that have a fixed appearance or change colour slowly this is especially problematic. Potential solutions include having fixed camouflage appearances that are optimized for matching elements of multiple backgrounds at once, or using disruptive coloration which involves high contrast markings breaking up the body outline (reviewed by Hughes et al., 2019). Additionally, individuals may be able to actively select substrates that match their own appearance (reviewed by Stevens & Ruxton, 2019). The latter approach has been appreciated for decades, especially in early studies of animals, particularly those focusing on moths and the mechanisms underpinning choices (e.g. Sargent, 1968; Grant & Howlett, 1988). More recent work, including on a range of invertebrates and vertebrates, has explored this area in the laboratory and field (see below). Such findings can help to explain phenotype–environment matches that occur on a fine microscale.

Animals are capable of making choices that facilitate concealment, but several key gaps remain to be addressed (see Stevens & Ruxton, 2019). First, it is not known how often choices are made

* Corresponding author.
E-mail address: martin.stevens@exeter.ac.uk (M. Stevens).

https://doi.org/10.1016/j.anbehav.2023.06.007

on an individual level based on animals' unique appearances, rather than choices that are species or morph specific. This is particularly pertinent in animals that show considerable intraspecific variation. Most research has either focused on species level choices or not determined whether choice also differs between individuals of different phenotypes. Second, when and why individuals show variation in choices and whether this reflects traits such as appearance, size, life stage and sex are almost untested. Third, the significance of substrate choice for camouflage in a changing world, such as for facilitating invasiveness or for coping with altered habitats, needs addressing, given that anthropogenic changes are impacting camouflaged species (Zimova et al., 2014; Banos-Villalba et al., 2018; Carter et al., 2020; Koneru & Caro, 2022). Here, we addressed these issues in a series of experiments on the highly variable shore crab, a species that has excellent camouflage and is extremely diverse in individual appearance (e.g. Price et al., 2019; Stevens et al., 2014b; Todd et al., 2006). This species also shows phenotype–environment matches, at even a microscale (m^2; Todd et al., 2012; Nokelainen et al., 2017).

Studies of moths have shown that individuals can make choices in line with their brightness at a species- and morph-specific level (e.g. Sargent et al., 1966; Kettlewell, 1955; Kang et al., 2012; reviewed by Stevens & Ruxton, 2019). More recently, work has revealed that, in species with continuous variation in appearance, choices also appear to be made at an individual level. For example, in birds, laboratory studies showed that Japanese quail, *Coturnix japonica*, choose nesting backgrounds in line with the appearance of their eggs (Lovell et al., 2013), and multiple species of wild nesting birds, such as plovers and nightjars, select backgrounds on which to nest based on individual egg or adult appearances to improve concealment (Stevens et al., 2017). Similar findings have been found in studies of wild lizards (Marshall et al., 2016), and experiments showing aspects of background choice have also been undertaken in fish, tadpoles, grasshoppers, and cuttlefish, although the results are not always clear cut (see Stevens & Ruxton, 2019). In crustaceans, Uy et al. (2017) demonstrated that individual pallid ghost crabs, *Ocypode pallidula*, choose lighter or darker sand based on their own individual brightness. Chameleon prawns, *Hippolyte varians*, which can change colour between red and green over a period of weeks to match different seaweed, actively select coloured backgrounds that best match their current appearance in the short term (Green et al., 2019). The mechanisms underpinning background choice are rarely tested but multiple nonexclusive processes may exist (Stevens & Ruxton, 2019). These include individuals directly comparing their own appearance to that of the background, preferring backgrounds that they are familiar with or have imprinted on, and linkage between genes controlling preferences and coloration. In many instances, animals either need to visually distinguish between potential backgrounds and choose accordingly, or rely on other sensory information (e.g. olfaction or texture) which act as a reliable proxy for appearance.

Shore crabs are a valuable species to assess the significance of individual-specific background choice. Like many crabs (Caro, 2018), they show consistent phenotype–environment associations (Stevens et al., 2014a; Todd et al., 2006, 2012), and are well camouflaged in a variety of habitats, using both disruptive coloration and background matching (Price et al., 2019). Variation in appearance is observed between adults and juveniles (Hogarth, 1978; Todd et al., 2006), with mature crabs being less diverse and more uniform in appearance, whereas juveniles are more striking and colourful and display high variation among individuals (Stevens et al., 2014a). Shore crabs are capable of changing brightness to better match the background in a few hours (Stevens et al., 2014b), yet these alterations are relatively small. Instead, the vast majority of their changes in appearance arise over a period of weeks, including during moult and through ontogeny (Carter et al., 2020; Nokelainen et al., 2019; Stevens, 2016). As such, crabs can change appearance over time to effectively match their overall visual environment (habitat), but colour pattern change is too slow and apparently lacking in pattern refinement to enable close matching on a short timescale. Given that crabs are very mobile, this creates a problem, especially for the diverse juveniles (adults may rely on a generalist camouflage strategy; Nokelaninen et al., 2019). In field studies, crabs show phenotype–environment associations at a microscale between appearance type and substrate (Nokelainen et al., 2017; Todd et al., 2012), and the logical explanation for what drives this is that individual crabs can behaviourally choose specific patches that match their appearance.

Here, we used shore crabs from a heterogeneous rock pool habitat to test for substrate preferences and how these relate to individual appearance. In a series of experiments, crabs were presented with two substrates of varying brightness and colour, (1) dark or light, (2) red or green and (3) backgrounds broadly resembling rock pools or mudflats, and were allowed to choose between them. We first determined any overall preferences for given substrates. Second, using image analysis, we quantified brightness and colour metrics to test for associations between substrate choice and individual crab appearance, and whether individual crabs chose backgrounds that best matched their appearance. We predicted that crabs would choose substrates that best enhanced their individual camouflage with regard to brightness and colour, but that the strength of such choices may depend on factors such as maturity and the specific substrates offered. For example, juvenile crabs, which tend to have more pronounced patterns, may show different choices to adults, and crabs in general may show preferences for substrates that they are more familiar with (e.g. backgrounds resembling rock pools over those resembling mudflats).

METHODS

Fieldwork took place around low tide between March and June 2019 at Gyllyngvase beach (50°8′39.42″N, -5°4′5.244″W) in Falmouth, U.K. Gyllyngvase beach was selected as it incorporates large areas of rock pool habitats and has been well studied in past experiments. The rock pool microhabitats are an ideal environment where species may benefit from choosing appropriate backgrounds (Nokelainen et al., 2017). All crabs were caught by haphazardly searching under rocks and through the substrate in a focal area, identified (Crothers, 1968), and used only once. Each crab was first placed in a white tray and dabbed with a cloth to remove water (Stevens et al., 2014b) for photographing (see below for photography and image analysis). Three experiments were conducted testing shore crab preferences between light and dark, red and green, and simulated rock pool and mudflat substrates (see below).

In experiments 1 and 2, a custom made 'Y' shaped decision chamber was constructed inside a tray (43 × 31 cm and 9.5 cm high) to test whether shore crabs actively choose substrates best matching their appearance (similar to Green et al., 2019) over a short duration of 10 min. Gravel was layered across each of the chamber arms ending where they intersected, leaving the grey plastic base of the chamber exposed below this point. Crabs were placed at the base of the Y chamber. Every minute the crab's location was noted, and at the end of the 10 min the substrate occupied the most was recorded as the substrate chosen. Since we recorded the behaviour in real time, and the background treatments were obvious, the data were not recorded double blind. However, any possibility of subconscious bias should be low since crab choices were clear and it was unambiguous when a crab had moved along one arm of the arena or the other. We only used data from crabs that made a choice, giving a total of 53 crabs sampled in experiment 1 and 46 crabs in experiment 2.

For experiment 1, dark and light substrates were used (Fig. 1a and b) to test preferences based on brightness and background matching since crabs vary substantially in how light and dark they are. For this experiment there was a brightness difference of 27% reflectance between the dark and light substrates. We predicted that darker crabs would tend to choose the dark substrate, lighter crabs would similarly show a preference for the light substrate, and this would as a result improve background matching. In experiment 2, red and green substrates were used (Fig. 1c and d) to test preferences based on colour and background matching, since the natural rocks and substrate of the rock pools are covered with red encrusting and green macroalgae (e.g. Green et al., 2019). For this experiment, the brightness difference between red and green was minimized and controlled at 4% reflectance. By doing so, choices should be based on colour not overall brightness (although we acknowledge that there is uncertainty regarding crab perception of luminance and colour; see Discussion). Although green and red may seem rather unnatural or bright colours, they are common seaweed hues in the environment, and we have previously found microscale habitat associations between crab colours and such substrates (Nokelainen et al., 2017). Although crabs do not vary as much in colour as for brightness and pattern, they do tend towards green and brown hues, with some being more reddish. We predicted that to enhance camouflage, crabs should choose red or green substrates based on their own colour.

In experiment 3, a rectangular tray (40 × 28 cm and 8.5 cm high) was prepared with the substrates poured evenly on each half of the container. Here, we aimed to allow a different type of choice that covered a larger area (albeit not greatly bigger) that crabs could move between and with additional elements that they would naturally encounter, such as shelter. A large grey stone was placed in each corner of one width of the container (one on each of the separate substrates) to provide refuge and shade from direct sunlight. Stones were bought from a marine approved retailer (Pets at Home), and were identical in size, shape and weight. We allowed crabs more time to make decisions than in the earlier two experiments and provided a shelter to improve the realism of the environments. The experiment was limited to 2 h to prevent potential changes in appearance made by crabs influencing the outcome of the experiment. Crabs were placed in the centre of the tray, positioned across both substrates, and left for 2 h. Every 10 min the crab's location was noted, and at the end of the 2 h the substrate occupied the most was recorded as the substrate choice. A total of 40 crabs were sampled in experiment 3. The gravel used as substrates was bought from Pets at Home (brands: Unipact and Pettex) and marine safe. All equipment was washed prior to and between

Figure 1. Examples of substrate types used in the experiments. Experiment 1: (a) dark and (b) light; experiment 2: (c) red and (d) green; experiment 3: (e) rock pool and (f) mudflat. Note that brightness of these images is not standardized.

trials and after experimental use to prevent lingering chemical or biological cues. Experiment 3 tested whether shore crabs actively choose more naturalistic backgrounds resembling either rock pool or mudflat (another commonly used habitat) substrates (Fig. 1e and f) that best match their appearance. The rock pool substrate consisted of gravel ranging from white to brown, approximately replicating the diverse and complex rock pool environment. The mudflat substrate used brown and green gravel added in a 2:1 ratio, respectively, for the purpose of mimicking the more uniform coloration of mudflats and green algae (see similar substrates in Nokelainen et al., 2019). These two substrates were of similar brightness with an average reflectance difference of 3%. We predicted that crabs would be more likely to choose the artificial rock pool substrate based on the environment from which they were sourced, but that crabs may also choose substrates that are a closer match to their own colour for improved camouflage.

After each trial individuals were measured for size (mm), maturity (adult/juvenile) and sex (male/female; where possible/large enough). Crab size was measured from the two widest points on the carapace using digital callipers. Individuals measuring 21 mm or less were classified as juveniles. Those that measured 22 mm or more were classified as adults and were then sexed by viewing their abdomen (Crothers, 1968). The crabs were measured after the experiment to prevent any stress or colour change affecting the crab's substrate choice. In all three experiments, the trays contained a layer of water to keep crabs wet and reduce potential stress and replicated a shallow intertidal area. After each trial, fresh sea water was replaced in the experimental container and the sampled crab was returned to where it was found. Any crabs that appeared to have eggs, were soft from a recent moult or had the barnacle parasite (Isaeva et al., 2005) were not used in the experiments.

Photography and Image Analysis

A digital Sony A7 DLSR camera, fitted with a 28–70 mm lens, was used for all photographs taken in RAW format (.ARW) with fixed zoom and aperture, and shutter speed varying for appropriate exposure. The camera was attached to a tripod and pointed downwards to photograph the crab's carapace and the six substrates. A grey standard was placed into each photo, which reflected a known amount of light equally at 18% between 400 and 750 nm (Stevens et al., 2007). This allowed images to be controlled for any changes in light conditions that occurred in the field due to weather conditions. Three images were taken in the human visible spectrum (400–750 nm) at a range of exposures (±3) to avoid under or overexposed images. We did not include UV images since crabs show little UV reflectance and their aquatic predators lack UV vision (Nokelainen et al., 2017).

To quantify crab appearance for brightness and colour, images first needed to be processed to produce reflectance values for shortwave (SW), mediumwave (MW) and longwave (LW) channels. We did not model predator vision owing to the substantial range of predators that crabs face (see Discussion). Reflectance values are measured on a scale ranging from 0% to 100%, where an image value of 655 on a 16-bit scale equals 100% reflectance. To produce these data, photographs were first uploaded in RawTherapee (open source from rawtherapee.com). For all crabs and substrates, an optimal exposed image was selected by viewing the RGB histogram. Overexposed images cause a loss of pixel data and therefore cannot be measured accurately (Troscianko & Stevens, 2015). Next, images were converted to multispectral images (MSPEC) using the MICA toolbox program Image J (Troscianko & Stevens, 2015). During this process, images were split into stacks of three images of relative wavebands SW, MW and LW. Images were standardized based on the 18% grey standard to control for light conditions between photos (Stevens et al., 2007; Troscianko & Stevens, 2015). Following this, regions of interest (ROIs) required for image analysis were selected and average reflectance measured. In the case of crab appearance, the area of the carapace was selected for ROIs, avoiding any specular reflectance. In the case of substrate appearance, a square area (1000 × 1000 pixels) was selected from the centre for ROIs. Multispectral images were processed through batch process imaging tools, producing SW, MW and LW reflectance values for the crabs and substrates.

Quantification of Brightness and Colour

Crab and substrate appearance were measured for brightness and colour. First, brightness was calculated (as in Stevens et al., 2013, 2014) to determine how dark or light an object was across the entire spectrum: brightness = (LW + MW + SW)/3. Next, measures were used to describe different colours in a reflectance colour space based on x, y coordinates based on standard equations for representing colours in a trichromatic (triangular) space (see Endler & Mielke, 2005; Stevens et al., 2009). Using x and y coordinates, the Euclidean distance between two objects can be calculated to provide a measure of colour difference (Endler & Mielke, 2005; Stevens et al., 2009).

To assess whether crabs chose backgrounds best matching their appearance in terms of brightness, the absolute difference between individual crab brightness and each potential substrate was calculated. This comparison determines which substrate provides the best match in terms of brightness, and hence which choice crabs should make. Similarly, a comparison of colour distance of the crabs and substrates determines which substrate is the best matching for colour. In both cases, crabs that chose substrates with the lowest values were correctly matched, and we determined whether the crab chose the substrate best matching its appearance.

Ethical Note

Shore crabs are not a protected species, and all work was conducted under approval from the University of Exeter Biosciences Ethics Committee (application 2019/eCORN001889). The field locations are publicly accessible; no further permits were needed. We ensured that crabs were kept no longer than necessary, returned to the same location where collected, kept in sea water and with minimal handling to reduce stress.

Statistical Analysis

Each individual crab was tested only once, in one of the experiments. Data were analysed using a binomial generalized linear model (GLM) to examine the potential impact of crab brightness (experiment 1), or crab colour distance (experiments 2 and 3), and maturity (as a categorical fixed factor for adults and juveniles) on substrate choice. The dependent variable was substrate choice and was modelled using a logit link function. The choice variable was binary, with options for dark/light, red/green and rockpool/mudflat. Appropriate sample sizes ($N = 40–53$) were used, normality was checked and data were log transformed where necessary for continuous independent variables. We also checked for multicollinearity. The substrates offered were the fixed factors. A full model containing all interactions was considered.

For brightness and colour differences between crabs and the substrates, a paired t test was used to determine whether crabs were significantly better matched to their chosen substrate compared to the alternative substrate. Finally, to determine whether crabs chose substrates that best matched their appearance, exact binomial proportion tests (binom.test function in RStudio) were used with frequency of chosen substrates compared to a proportion of 0.5 (Green et al., 2019). All analyses were conducted in the statistical program R (R Development Core Team 2016).

RESULTS

Experiment 1: Dark and Light

A total of 53 crabs were sampled in experiment 1: 19 adults and 34 juveniles. Crabs showed preferences that were overall in favour of the dark over the light substrate (Fig. 2a), and individuals that chose dark substrates were significantly lower in brightness than crabs that chose light substrates (binomial GLM: $Z = 3.033$, $P = 0.002$; Fig. 2b). There was no relationship between chosen substrates and crab maturity (binomial GLM: $Z = -1.148$, $P = 0.251$). Overall, crabs were significantly better matched to their chosen substrate than to the alternative substrate (paired t test: $t_{52} = -3.988$, $P < 0.001$), and a significant proportion of crabs chose substrates best matching their own appearance (exact binomial test: proportion = 0.72, $N = 53$, $P = 0.002$; Fig. 2c) in terms of brightness.

Experiment 2: Red and Green

A total of 46 crabs were used in experiment 2: 24 adults and 22 juveniles. Crabs showed no evidence of substrate choice (Fig. 3a), and there was no difference in the choices that crabs made linked to differences in the background they matched better, not least because the average match to both backgrounds was similar (binomial GLM: $Z = 1.628$, $P = 0.104$). There was no relationship between chosen substrates and crab maturity (binomial GLM: $Z = -1.148$, $P = 0.251$). Overall, crabs were not significantly better matched to their chosen substrate than to the alternative substrate (paired t test: $t_{45} = -1.186$, $P = 0.242$; Fig. 3b), and there was no evidence that crabs chose substrates with the colour best matching their own appearance (exact binomial test: proportion = 0.54, $N = 46$, $P = 0.659$; Fig. 3c).

Experiment 3: Rock Pool and Mudflat

A total of 40 crabs were caught in experiment 3: 11 adults and 29 juveniles. Overall, crabs showed a preference for the rock pool substrate (Fig. 4a), and there was a relationship between chosen substrate and colour distance, with crabs choosing the substrate that they on average matched better (binomial GLM: $Z = -2.29$, $P = 0.022$). There was no relationship between chosen substrates and crab maturity (binomial GLM: $Z = -0.703$, $P = 0.482$). Overall, crabs were significantly better matched to their chosen substrate than to the alternative substrate (paired t test: $t_{39} = -2.839$, $P = 0.007$; Fig. 4b), and a significant proportion of crabs chose substrates with colours closest to their own (exact binomial test: proportion = 0.70, $N = 40$, $P = 0.017$; Fig. 4c).

DISCUSSION

This series of experiments tested the preferred substrates of shore crabs and the relationship between crab appearance and the background selected. Crabs were used in one of three experiments involving choices between dark and light, red and green, and approximated rock pool and mudflat colours. Using image analysis to quantify brightness and colour of both the crabs and experimental backgrounds, the match between crabs and substrates for active background matching was tested. In experiment 1 (dark and light), crabs tended to choose the dark substrate, but overall crabs chose substrates with a brightness best matching their own appearance. Crabs of brighter appearance were more likely to choose the white substrate than were darker crabs. In experiment 2 (red and green), crabs did not show a distinct preference for either red or green, and crabs did not choose substrates best matching their own appearance. In experiment 3 (rock pool and mudflat), the majority of crabs chose the rock pool substrate and this was the best matching to their own measured colour. Our study therefore shows that shore crabs can actively select backgrounds, and at least in some cases they appear capable of choosing backgrounds best matching their own specific appearance.

In experiment 1, crabs showed an overall preference towards the dark substrate. Individuals that chose the dark substrate were

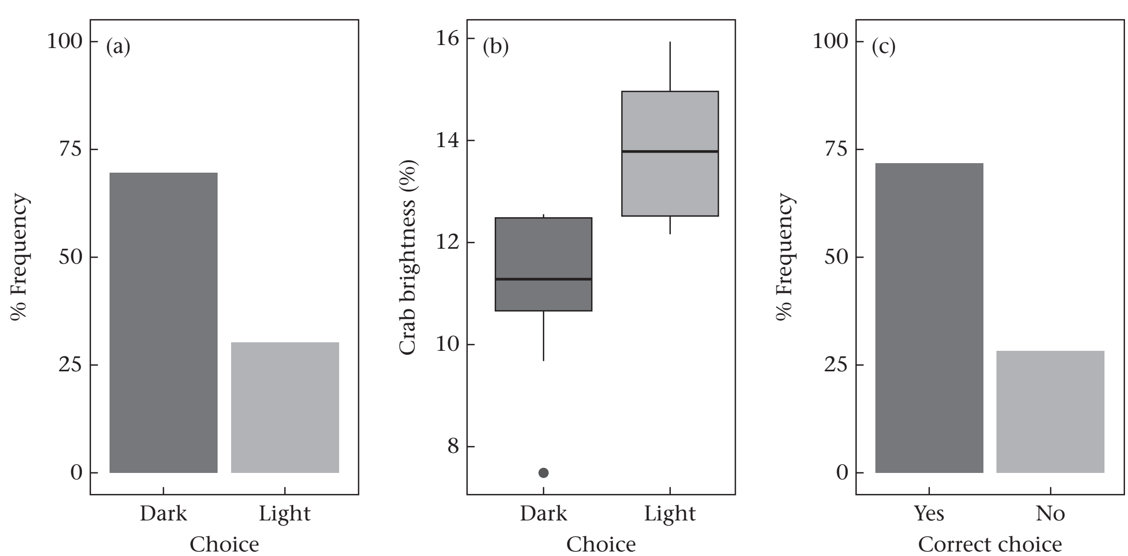

Figure 2. Experiment 1. (a) The frequency of substrates chosen by shore crabs ($N = 53$) presented with two substrate types: dark ($N = 37$) and light ($N = 16$). (b) The brightness (overall reflectance) of shore crabs that chose the dark or the light substrate. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circle is an outlier. (c) The frequency of substrates that were correctly (matching, $N = 38$) or incorrectly (nonmatching, $N = 15$) chosen by shore crabs.

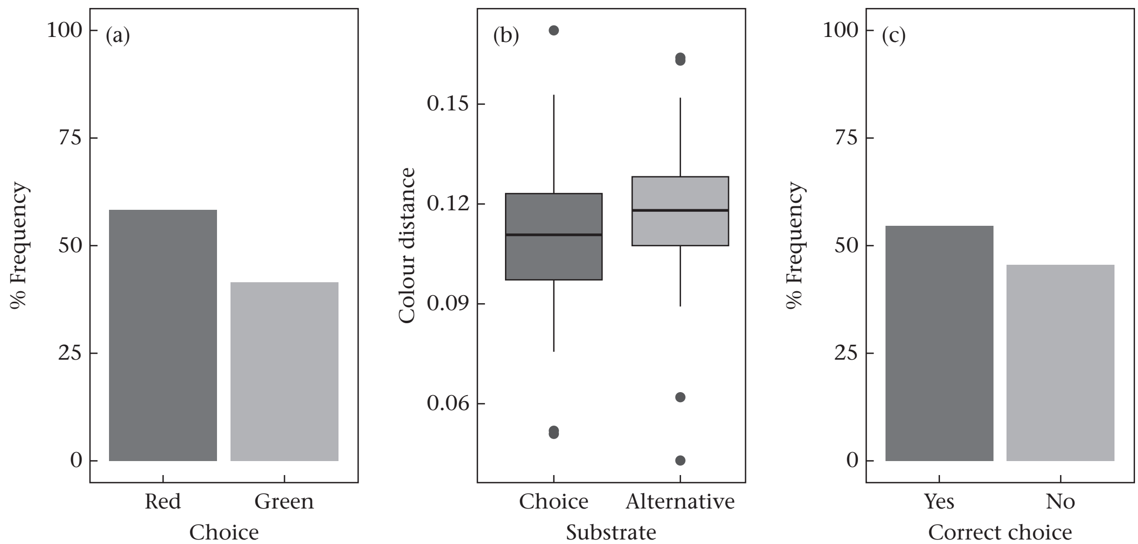

Figure 3. Experiment 2. (a) The frequency of substrates chosen by shore crabs ($N = 46$) presented with two substrate types: red ($N = 27$) and green ($N = 19$). (b) The colour distance of shore crabs on their chosen substrate and the alternative substrate. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are outliers. (c) The frequency of substrates that were correctly (matching, $N = 25$) or incorrectly (nonmatching, $N = 21$) chosen by shore crabs.

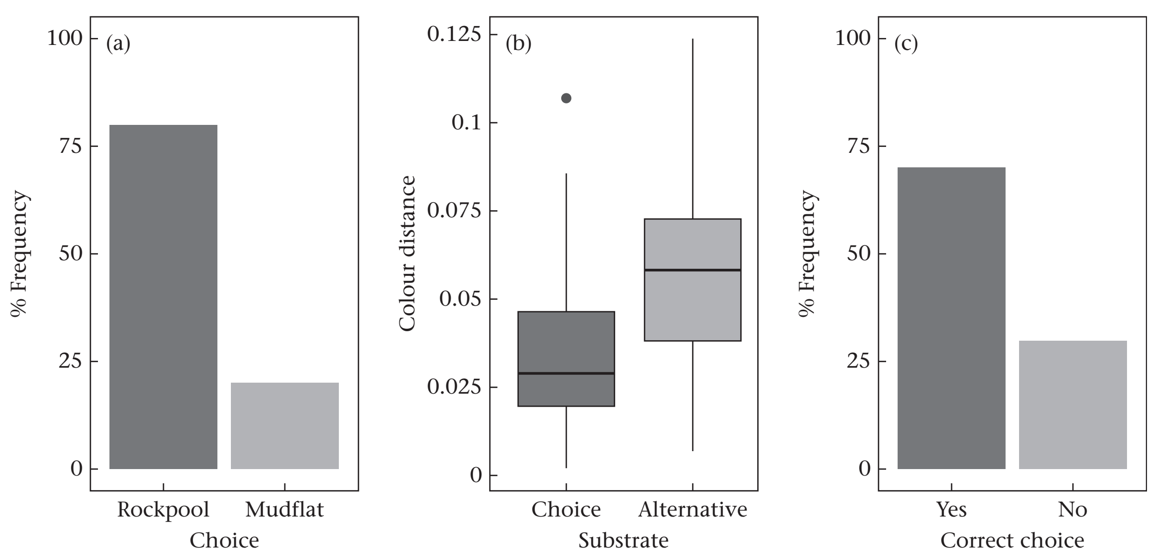

Figure 4. Experiment 3. (a) The frequency of substrates ($N = 40$) chosen by shore crabs presented with two substrate types: rock pool ($N = 32$) and mudflat ($N = 8$). (b) The colour distance of shore crabs on their chosen substrate and the alternative substrate. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circle is an outlier. (c) The frequency of substrates that were correctly (matching, $N = 28$) or incorrectly (nonmatching, $N = 12$) chosen by shore crabs.

significantly better matched to this. However, there was a clear difference in individual crab brightness between those that chose the dark substrate and those that chose the light substrate. Darker crabs tended to choose the dark substrate, whereas crabs that chose the white substrate were lighter in colour. However, the result is not clear cut since crabs that chose the light substrate were still generally better matched to the dark substrate. None the less, our findings show some degree of difference in choice in a manner that is in accordance with individual crab appearance. Overall, crabs could discriminate between light and dark and effectively chose backgrounds that matched their appearance for brightness, albeit with some variation. Similar to these results, ghost crabs (Uy et al., 2017), rock gobies, *Gobius paganellus* (Smithers et al., 2018) and azure sand grasshoppers, *Sphingonotus azurescens* (Edelaar et al., 2019; Camacho et al., 2020), choose backgrounds that best match their appearance in terms of brightness. In the ghost crabs, preferences were clearly linked to how dark or light individuals were, whereas in other systems, such as gobies, preferences are more general across all individuals for the darker background. Most comprehensively, work on grasshoppers in urban areas of light and dark substrates shows not only that individuals choose to rest on substrates that better match their brightness, but also that manipulation of individual brightness changes the preferences of grasshoppers in both laboratory and field experiments (Edelaar et al., 2019; Camacho et al., 2020). Our results here are perhaps most similar to findings in gobies, in that most individuals preferred the dark substrate, with more limited evidence that crabs showed individual level preferences rather than a species/population-wide preference for darker substrates. However, given that most crabs are relatively dark, this may not have been the most effective test of this. Furthermore, crabs that chose the lighter substrate were lighter than those that chose the darker substrate. In fact, seven of the 10 brightest crabs chose the light substrate, which does indicate a link between individual appearance and preference. We did not have very many crabs that were especially bright (e.g. cream or white) in our study so it would be worth exploring the preferences of those rarer individuals, and with backgrounds that are less extreme in brightness.

In experiment 2, there was no obvious preference for either red or green substrate. There is uncertainty regarding shore crab vision, but they likely have only one or two receptors and may lack the ability to discriminate green from red, owing to missing LW or MW cone sensitivities (Martin & Mote, 1982), unless they use brightness as a proxy. Overall, the proportion of correct colour background matching was not significant and appeared random. It may be that, since shore crabs are so diverse in colour and pattern, neither background provided camouflage advantages or disadvantages. This differs from other research in another marine invertebrate, the chameleon prawn, which accurately choose between red and green backgrounds, but in which individuals come in clearly distinct red and green forms (Green et al., 2019). Shore crabs are more diverse in colour and patterns and use a wider range of the complex rock pool substrates as a background to match. Highly patterned rock pool crabs also rely more on disruptive coloration than background matching (Price et al., 2019), and so fine-scale colour matching may not be important to them, compared to matching more pronounced differences in overall brightness.

In experiment 3, a large proportion of crabs preferred the (mock) rock pool substrate over the mudflat substrate. Crabs that chose the rock pool substrate were significantly better matched to their substrates in terms of colour than those that selected the mudflat substrate. The green and brown mixed gravel (mudflat substrate) is less complex; therefore, if crabs do have limited colour vision, as suggested above, it may be that they were observing a uniform (mudflat) environment compared to a complex (rock pool substrate) environment that had a range of natural-coloured stones. As with experiment 1, crabs were generally much closer in colour to the rock pool substrate than to the mudflat one, and crabs that chose the mudflat substrate would have been better off choosing the alternative. Therefore, we cannot say with confidence that choices are based on an overall species/population or site trait shared by most crabs regardless of individual colour, as opposed to individual-specific preferences linked to their own appearance.

Much research on shore crab camouflage provides evidence for phenotype–environment matching. For example, crabs from different habitat types, and different substrates at a microscale, show consistent differences in appearance and this is linked to camouflage matching and disruptive coloration (Nokelainen et al., 2018, 2019; Price et al., 2019; Stevens et al., 2014; Todd et al., 2006, 2012). Our results show that crabs do choose backgrounds when given a choice, and that the choices they make are those that should, on the whole, improve their match to the substrate. On a larger scale, this should enable crabs to remain within a habitat or site to which their coloration is broadly tuned. Considering the multitude of evidence showing crabs can alter their appearance to match their current background, especially over a period of weeks (Carter et al., 2020; Nokelainen et al., 2019; Powell, 1964; Stevens, 2016; Stevens et al., 2014b), they will often show appearances linked to their habitat. The slow rate of appearance change in this species then means that active background choice would be valuable in maintaining this camouflage in the medium term.

We did not find clear evidence that background choice was more evident in either juveniles or adults. Further work with more equal and greater sample sizes is needed to explore this further. Once crabs mature and undergo ontogenetic changes in colour and move more freely among habitats, adults appear to adopt more generalist camouflage colours (Nokelainen et al., 2019). Their predation risk may also be lower. These two factors may reduce the need to select matching backgrounds. By contrast, on a small scale, as juvenile crabs move around within a habitat, substrate preferences on a microscale linked to appearance would be beneficial, and studies have reported such correlations in the field (Nokelainen et al., 2017; Todd et al., 2012). Although our study did not show clear evidence of choices linked to unique crab appearances, we did find hints that crabs are capable of this. Indeed, other crabs have been shown to have this ability, albeit in species with less intraspecific variation (Uy et al., 2017).

While our study shows that shore crabs use behaviour to facilitate camouflage, there are areas left to explore. First, future experiments should test crabs showing a greater and more distinct contrast in brightness and colour. For example, we did not use many predominantly white crabs, yet they should in principle prefer lighter backgrounds. Shore crabs, in particular those from rocky shores and juveniles, can occur in very light forms and be marked with bright patterns (Stevens et al., 2014a), so there should be substantial benefits to those individuals in selecting lighter substrates. In addition, testing differences in choices between crabs from other locations and habitats beyond rock pools, such as mudflat and mussel bed crabs, would be valuable. We would expect mudflat crabs to prefer the mudflat substrate used here, and crabs to show general preferences for backgrounds closest to their habitat of origin. In this study we also used artificial substrates, largely to better control potential other factors such as familiarity with the substrate and other (e.g. odour or texture) cues that may be present. Artificial substrates allow for tighter control of appearance too. However, while we doubt that the general nature of choices would change if using natural substrates, it would be valuable to test these in future, as well as a greater range of them to better explore the nature and extent of choices. Research should also explore the mechanisms through which crabs are able to make their choices (Stevens & Ruxton, 2019), whether it be genetically based, learnt or via imprinting, or whether crabs can somehow compare their own appearance to the substrate itself directly. Additionally, the question of determining to what extent background choice improves camouflage and increases survival chances needs further addressing. It is evident that background choice behaviour can facilitate camouflage, but to what extent animals make background choices and the survival advantage conferred need exploration.

In this study we did not model predator vision but instead used objective measures based on reflectance. Crabs are known to have a substantial range of predators, from birds with both a violet and ultraviolet system to mono-, di- and trichromatic fish predators, cephalopods and other crabs (see Crothers, 1968). Modelling such a huge range of visual systems was beyond our study, and in previous work when we have compared fish and bird visual perception of crabs, we have not identified substantial differences that would indicate that camouflage efficacy should vary greatly among these groups (Nokelainen et al., 2017), and in fact we would expect crab camouflage to be effective to the suite of predators they face. What also matters is how crabs themselves see the backgrounds, yet shore crab vision is still not entirely well understood (see above). None the less, more comprehensive work, particularly at different water depths and habitats, would be valuable in testing if, and when, differences in predator vision may be important. It would also be valuable to model how crab camouflage varies with detection distances by predators, in line with predator acuity, alongside issues such as light attenuation with water depth, turbidity, distance and predator colour vision.

Background matching provides a clear understanding of the selection pressures faced by many species and how animals adapt to different environments. Individuals that can successfully use behaviour to select best-matching backgrounds would likely lower their predation risk (Théry & Casas, 2002; Ahnesjö & Forsman, 2006), and this trait could even lead to high population densities resulting in intraspecific competition and displacement (Diffendorfer, 1998; Duarte et al., 2016). Modifying behaviour could also be an important factor in facilitating successful invasions of species outside their natural range, and shore crabs are a major invader in many parts of the world (Stevens & Ruxton, 2019), playing a fundamental role in the success of a species in exploiting a variety of different environments. These abilities could help explain why some species are so successfully invasive outside their natural range. By contrast, choice of backgrounds could help individuals of other species overcome issues related to habitat and climate change, although there has been little study or evidence of this so far (but see for example Zimova et al., 2014). However, predicting changes caused by anthropogenic impacts is challenging, not least as there are many interacting issues, from noise and chemical pollution to invasive seaweeds, all of which are present at our study site. In shore crabs, both noise and chemical pollution have been shown to impact their coloration and antipredator behaviours (e.g. Carter et al., 2020; Chabenat et al., 2019; Rising et al., 2022; Wale et al., 2013). The ability to change colour and select optimal resting spots may prove to be a vital trait for animals facing changes to habitats (Delhey & Peters, 2017). Yet this remains to be properly tested, even though the visual environment at our study site (and others) is changing rapidly owing to the spreading of a suite of non-native seaweeds. With the highlighted substantial gaps in our knowledge of the role of background choice in camouflage, ample room exists for further exploration and evolutionary understanding, both in a pure and in a conservation context.

Author Contributions

L.T. and M.S. conceived the overall study and main ideas; L.T. undertook the experiments and analysis with input from M.S.; L.T. wrote the initial draft of the paper with contributions and editing from M.S.

Data Availability

Data from each experiment are available as a supplementary file.

Declaration of Interest

We declare no competing interests.

Acknowledgments

We thank Tim Caro and an anonymous referee for helpful comments on the manuscript.

Supplementary material

Supplementary material associated with this article is available in the online version at https://doi.org/10.1016/j.anbehav.2023.06.007.

References

Ahnesjö, J., & Forsman, A. (2006). Differential habitat selection by pygmy grasshopper color morphs; interactive effects of temperature and predator avoidance. *Evolutionary Ecology, 20*, 235–257.

Baños-Villalba, A., Quevedo, P. D., & Edelaar, P. (2018). Positioning behavior according to individual color variation improves camouflage in novel habitats. *Behavioral Ecology, 29*, 404–410.

Boratyński, Z., Brito, J. C., Campos, J. C., Cunha, J. L., Granjon, L., Mappes, T., Ndiaye, A., Rzebik-Kowalska, B., & Serén, N. (2017). Repeated evolution of camouflage in speciose desert rodents. *Scientific Reports, 7*, 3522.

Briffa, M., Haskell, P., & Wilding, C. (2008). Behavioural colour change in the hermit crab *Pagurus bernhardus*: Reduced crypticity when the threat of predation is high. *Behaviour, 145*, 915–929.

Camacho, C., Sanabria-Fernández, A., Baños-Villalba, A., & Edelaar, P. (2020). Experimental evidence that matching habitat choice drives local adaptation in a wild population. *Proceedings of the Royal Society B: Biological Sciences, 287*, Article 20200721.

Caro, T. (2018). The functional significance of coloration in crabs. *Biological Journal of the Linnean Society, 124*, 1–10.

Caro, T., Sherratt, T. N., & Stevens, M. (2016). The ecology of multiple colour defences. *Evolutionary Ecology, 30*, 797–809.

Carter, E. E., Tregenza, T., & Stevens, M. (2020). Ship noise inhibits colour change, camouflage, and anti-predator behaviour in shore crabs. *Current Biology, 30*, R211–R212.

Chabenat, A., Bellanger, C., Jozet-Alves, C., & Knigge, T. (2019). Hidden in the sand: Alteration of burying behaviour in shore crabs and cuttlefish by antidepressant exposure. *Ecotoxicology and Environmental Safety, 186*, Article 109738.

Crothers, J. H. (1968). The biology of the shore crab *Carcinus maenas* (L.): The background anatomy, growth and life history. *Field Studies, 2*, 407–434.

Delhey, K., & Peters, A. (2017). Conservation implications of anthropogenic impacts on visual communication and camouflage. *Conservation Biology, 31*, 30–39.

Diffendorfer, J. E. (1998). Testing models of source-sink dynamics and balanced dispersal. *Oikos, 81*, 417–433.

Duarte, R. C., Flores, A. A. V., & Stevens, M. (2017). Camouflage through colour change: mechanisms, adaptive value, and ecological significance. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*, Article 20160342.

Duarte, R. C., Stevens, M., & Flores, A. A. V. (2016). Shape, colour plasticity, and habitat use indicate morphspecific camouflage strategies in a marine shrimp. BMC. *Evolutionary Biology, 16*, 218.

Edelaar, P., Baños-Villalba, A., Quevedo, D. P., Escudero, G., Bolnick, D. I., & Jordán-Andrade, A. (2019). Biased movement drives local cryptic coloration on distinct urban pavements. *Proceedings of the Royal Society B: Biological Sciences, 286*, Article 20191343.

Endler, J. A. (1978). A predator's view of animal color patterns. *Evolutionary Biology, 11*, 319–364.

Endler, J. A. (1984). Progressive background matching in moths, and a quantitative measure of crypsis. *Biological Journal of the Linnean Society, 22*, 187–231.

Endler, A. O., & Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society, 86*, 405–431.

Grant, B., & Howlett, R. J. (1988). Background selection by the peppered moth (*Biston betularia* Linn.): Individual differences. *Biological Journal of the Linnean Society, 33*, 217–232.

Green, S., Duarte, R. C., Kellett, E., Alagaratnam, N., & Stevens, M. (2019). Colour change and behavioural choice facilitate chameleon prawn camouflage against different seaweed backgrounds. *Communications Biology, 2*, 230.

Hogarth, P. (1978). Variation in the carapace pattern of juvenile *Carcinus maenas*. *Marine Biology, 44*, 337–343.

Hughes, A., Liggins, E., & Stevens, M. (2019). Imperfect camouflage: How to hide in a variable world. *Proceedings of the Royal Society B: Biological Sciences, 286*, Article 20190646.

Isaeva, V. M., Dolganov, S., & Shukalyuk, A. (2005). Rhizocephalan Barnacles – Parasites of commercially important crabs and other decapods. *Russian Journal of Marine Biology, 31*, 215–220.

Kang, C. K., Moon, J. Y., Lee, S. I., & Jablonski, P. G. (2012). Camouflage through an active choice of a resting spot and body orientation in moths. *Journal of Evolutionary Biology, 25*, 1695–1702.

Kettlewell, H. B. D. (1955). Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. *Nature, 175*, 943–944.

Koneru, M., & Caro, T. (2022). Animal coloration in the Anthropocene. *Frontiers in Ecology and Evolution, 10*, Article 857317.

Lovell, P. G., Ruxton, G. D., Langridge, K. V., & Spencer, K. A. (2013). Individual quail select egg-laying substrate providing optimal camouflage for their egg phenotype. *Current Biology, 23*, 260–264.

Magellan, K., & Swartz, E. R. (2013). Crypsis in a heterogeneous environment: Relationships between changeable polymorphic colour patterns and behaviour in a galaxiid fish. *Freshwater Biology, 58*, 793–799.

Marshall, K. L. A., Philpot, K. E., & Stevens, M. (2016). Microhabitat choice in island lizards enhances camouflage against avian predators. *Scientific Reports, 6*, Article 19815.

Martin, F., & Mote, M. (1982). Color receptors in marine crustaceans: A second spectral class of retinular cell in the compound eyes of *Callinectes* and *Carcinus*. *Journal of Comparative Physiology, 145*, 549–554.

Merilaita, S., & Stevens, M. (2011). Crypsis through background matching. In M. Stevens, & S. Merilaita (Eds.), *Animal camouflage: Mechanisms and function* (pp. 17–33). Cambridge University Press.

Michalis, C., Scott-Samuel, N. E., Gibson, D. P., & Cuthill, I. C. (2017). Optimal background matching camouflage. *Proceedings of the Royal Society B: Biological Sciences, 284*, Article 20170709.

Nokelainen, O., Hubbard, N., Lown, A. E., Wood, L. E., & Stevens, M. (2017). Through predators' eyes – phenotype-environment associations in shore crab coloration at different spatial scales. *Biological Journal of the Linnean Society, 122*, 738–751.

Nokelainen, O., Maynes, R., Mynott, S., Price, N., & Stevens, M. (2019). Improved camouflage through ontogenetic colour change confers reduced detection risk in shore crabs. *Functional Ecology, 33*, 654–669.

Nokelainen, O., Stevens, M., & Caro, T. (2018). Colour polymorphism in the coconut crab (*Birgus latro*). *Evolutionary Ecology, 32*, 75–88.

Powell, B. L. (1964). The responses of the chromatophores of *Carcinus maenas* (L. 1758) to light and temperature. *Crustaceana, 4*, 93–102.

Price, N., Green, S., Troscianko, J., Tregenza, T., Stevens, M., & Harvey, I. F. (2019). Background matching and disruptive coloration as habitat-specific strategies for camouflage. *Scientific Reports, 8*, 7840.

R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rising, K., Hardege, J., Tregenza, T., & Stevens, M. (2022). Anthropogenic noise may impair the mating behaviour of the Shore Crab *Carcinus maenas*. *PLoS One, 17*, Article e0276889.

Rosenblum, E. B. (2006). Convergent evolution and divergent selection: Lizards at the White Sands ecotone. *American Naturalist, 167*, 1–15.

Rowland, H. M., Speed, M. P., Ruxton, G. D., Edmunds, M., Stevens, M., & Harvey, I. F. (2007). Countershading enhances cryptic protection: An experiment with wild birds and artificial prey. *Animal Behaviour, 74*, 1249–1258.

Ruxton, G. D., Speed, M. P., & Sherratt, T. N. (2004). *Avoiding attack: The evolutionary ecology of crypsis, warning coloration and mimicry*. Oxford University Press.

Sargent, T. D. (1966). Background selections of geometrid and noctuid moths. *Science, 154*, 1674–1675.

Sargent, T. D. (1968). Cryptic moths: Effects on background selections of painting the circumocular scales. *Science, 159*, 100–101.

Smithers, S. P., Rooney, R., Wilson, A., & Stevens, M. (2018). Rock pool fish use a combination of colour change and substrate choice to improve camouflage. *Animal Behaviour, 144*, 54–65.

Stevens, M. (2016). Color change, phenotypic plasticity, and camouflage. *Frontiers in Ecology and Evolution, 4*, 51.

Stevens, M., Broderick, A. C., Godley, B. J., Lown, A. E., Troscianko, J., Weber, N., & Weber, S. B. (2015). Phenotype-environment matching in sand fleas. *Biology Letters, 11*, Article 20150494.

Stevens, M., Lown, A. E., & Denton, A. M. (2014). Rockpool gobies change colour for camouflage. *PLoS One, 9*, Article e110325.

Stevens, M., Lown, A. E., & Wood, L. E. (2014a). Camouflage and individual variation in shore crabs (*Carcinus maenas*) from different habitats. *PLoS One, 9*, Article e115586.

Stevens, M., Lown, A. E., & Wood, L. E. (2014b). Color change and camouflage in juvenile shore crabs *Carcinus maenas*. *Frontiers in Ecology and Evolution, 2*, 1–14.

Stevens, M., & Merilaita, S. (2009). Animal camouflage: Current issues and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*, 423–427.

Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C., & Troscianko, T. S. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society, 90*, 211–237.

Stevens, M., Pei Rong, C., & Todd, P. A. (2013). Colour change and camouflage in the horned ghost crab *Ocypode ceratophthalmus*. *Biological Journal of the Linnean Society, 109*, 257–270.

Stevens, M., & Ruxton, G. (2019). The key role of behaviour in animal camouflage. *Biological Reviews, 94*, 116–134.

Stevens, M., Stoddard, C., Mary, & Higham James, P. (2009). Studying primate color: Towards visual system-dependent methods. *International Journal of Primatology, 30*, 893–917.

Stevens, M., Troscianko, J., Wilson-Aggarwal, J., & Spottiswoode, C. N. (2017). Improvement of individual camouflage through background choice in ground-nesting birds. *Nature Ecology and Evolution, 1*, 1325–1333.

Théry, M., & Casas, J. (2002). Predator and prey views of spider camouflage. *Nature, 415*, 133.

Todd, P. A., Briers, R. A., Ladle, R. J., & Middleton, F. (2006). Phenotype-environment matching in the shore crab (*Carcinus maenas*). *Marine Biology, 148*, 1357–1367.

Todd, P. A., Oh, J., Loke, L. H. L., & Ladle, R. J. (2012). Multi-scale phenotype-substrate matching: Evidence from shore crabs (*Carcinus maenas* L.). *Ecological Complexity, 12*, 58–62.

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox – A free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution, 6*, 1320–1331.

Uy, F. M. K., Ravichandran, S., Patel, K. S., Aresty, J., Aresty, P. P., Audett, R. M., Chen, K., Epple, L., Jeffries, S. F., Serein, G. N., Tullis-Joyce, P., & Uy, J. A. C. (2017). Active background choice facilitates crypsis in a tropical crab. *Biotropica, 49*, 365–371.

Wale, M. A., Simpson, S. D., & Radford, A. N. (2013). Noise negatively affects foraging and antipredator behaviour in shore crabs. *Animal Behaviour, 86*, 111–118.

Walton, O. C., & Stevens, M. (2018). Avian vision models and field experiments determine the survival value of peppered moth camouflage. *Communications Biology, 1*, 118.

Zimova, M., Mills, L. S., Lukacs, P. M., & Mitchell, M. S. (2014). Snowshoe hares display limited phenotypic plasticity to mismatch in seasonal camouflage. *Proceedings of the Royal Society B: Biological Sciences, 281*, Article 20140029.